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SUPPLEMENTAL MATERIAL

FOR

The Architecture of the Cytoplasmic Region of Type III Secretion Systems

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Supplementary Tables S1 & S2

References

Supplementary Figures Legends S1-S3

Supplementary Figures S1-S3

TABLE S1. *Shigella flexneri* strains used in this study

Strain	Genotype (strain; plasmid)	Reference
WT	Wild-type M90T, serotype 5a	(1)
WT for minicells	WT; pBAD24 <i>Salmonella ftsZ</i>	This study and (2)
WT/FLAG_ <i>mxkK</i>	WT; pUC19 <i>mxkK</i> with N-terminal FLAG	This study
WT/ <i>spa33</i> _FLAG	WT; pUC19 <i>spa33</i> with C-terminal FLAG	This study
WT/FLAG_ <i>spa13</i>	WT; pUC19 <i>spa13</i> with N-terminal FLAG	This study
WT/FLAG_ <i>spa47</i>	WT; pUC19 <i>spa47</i> with N-terminal FLAG	This study
Δ <i>mxkK</i>	WT with deletion of <i>mxkK</i>	(3)
Δ <i>mxkK</i> /FLAG_ <i>mxkK</i>	Δ <i>mxkK</i> ; pUC19 <i>mxkK</i> with N-terminal FLAG	This study
Δ <i>mxkK</i> / <i>spa33</i> _FLAG	Δ <i>mxkK</i> ; pUC19 <i>spa33</i> with C-terminal FLAG	This study
Δ <i>mxkK</i> /FLAG_ <i>spa13</i>	Δ <i>mxkK</i> ; pUC19 <i>spa13</i> with N-terminal FLAG	This study
Δ <i>mxkK</i> /FLAG_ <i>spa47</i>	Δ <i>mxkK</i> ; pUC19 <i>spa47</i> with N-terminal FLAG	This study
Δ <i>mxkN</i>	WT with deletion of <i>mxkN</i>	(3)
Δ <i>mxkN</i> / <i>mxkN</i>	Δ <i>mxkN</i> ; pJN38	(3)
Δ <i>mxkN</i> /FLAG_ <i>mxkK</i>	Δ <i>mxkN</i> ; pUC19 <i>mxkK</i> with N-terminal FLAG	This study
Δ <i>mxkN</i> / <i>spa33</i> _FLAG	Δ <i>mxkN</i> ; pUC19 <i>spa33</i> with C-terminal FLAG	This study
Δ <i>mxkN</i> /FLAG_ <i>spa13</i>	Δ <i>mxkN</i> ; pUC19 <i>spa13</i> with N-terminal FLAG	This study
Δ <i>mxkN</i> /FLAG_ <i>spa47</i>	Δ <i>mxkN</i> ; pUC19 <i>spa47</i> with N-terminal FLAG	This study
Δ <i>spa33</i>	WT serotype 2a with deletion of <i>spa33</i>	(4)
Δ <i>spa33</i> /FLAG_ <i>mxkK</i>	Δ <i>spa33</i> ; pUC19 <i>mxkK</i> with N-terminal FLAG	This study
Δ <i>spa33</i> / <i>spa33</i> _FLAG	Δ <i>spa33</i> ; pUC19 <i>spa33</i> with C-terminal FLAG	This study
Δ <i>spa33</i> /FLAG_ <i>spa13</i>	Δ <i>spa33</i> ; pUC19 <i>spa13</i> with N-terminal FLAG	This study
Δ <i>spa33</i> /FLAG_ <i>spa47</i>	Δ <i>spa33</i> ; pUC19 <i>spa47</i> with N-terminal FLAG	This study
Δ <i>spa13</i>	WT with deletion of <i>spa13</i>	(5)

$\Delta spa13/FLAG_mxiK$	$\Delta spa13$; pUC19 <i>mxiK</i> with N-terminal FLAG	This study
$\Delta spa13/spa33_FLAG$	$\Delta spa13$; pUC19 <i>spa33</i> with C-terminal FLAG	This study
$\Delta spa13/FLAG_spa13$	$\Delta spa13$; pUC19 <i>spa13</i> with N-terminal FLAG	This study
$\Delta spa13/FLAG_spa47$	$\Delta spa13$; pUC19 <i>spa47</i> with N-terminal FLAG	This study
$\Delta spa47$	WT with deletion of <i>spa47</i>	(3)
$\Delta spa47/FLAG_mxiK$	$\Delta spa47$; pUC19 <i>mxiK</i> with N-terminal FLAG	This study
$\Delta spa47/spa33_FLAG$	$\Delta spa47$; pUC19 <i>spa33</i> with C-terminal FLAG	This study
$\Delta spa47/FLAG_spa13$	$\Delta spa47$; pUC19 <i>spa13</i> with N-terminal FLAG	This study
$\Delta spa47/FLAG_spa47$	$\Delta spa47$; pUC19 <i>spa47</i> with N-terminal FLAG	This study

TABLE S2. Primer sequences used in this study^{*}

Primer	Sequence
MxiK_FLAG_For	5'- AGTCTCTAGACAAGAGAAACAAATATGGACTACAAGGACGACGATGACAAGATAAGA ATGGATGGAAT-3'
MxiK_Rev	5'-AGTCGGATCCTCATAGGCATGATGTCTGG-3'
Spa13_FLAG_For	5'- AGTCTCTAGAAGGAGGTTATTGGTGGACTACAAGGACGACGATGACAAGAAACAATTA GATAAGG-3'
Spa13_Rev	5'-AGTCGGATCCTTATCTAATGCCATACTTC-3'
Spa47_FLAG_For	5'- AGTCTCTAGATGGGGTCTTATAATCAATGGACTACAAGGACGACGATGACAAGAGCTAT ACAAAATTGCTC-3'
Spa47_Rev	5'-AGTCGGATCCTTATCTAATTGTTTCACCA-3'
Spa33_FLAG_For	5'- AGTCTCTAGAACAGAGTGAAGAAGAATGGACTACAAGGACGACGATGACAAGCTAAG AATTAAACATTTT-3'
Spa33_Rev	5'-AGTCGGATCCTTACTCCTTTACCATCCAA-3'
Spa33_For	5'-AGTCTCTAGAACAGAGTGAAGAAGAATGCTAAGAATTAAACATTTT-3'
Spa33_FLAG_Rev	5'-AGTCGGATCCTTACTTGTCATCGTCGTCCTTGTAAGTCCTCCTTTACCATCCAAGA-3'

*Underlined capital letters represent restriction endonuclease sites generated to facilitate cloning. Italic capital letters represent FLAG sequence.

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SUPPLEMENTARY FIGURE LEGENDS

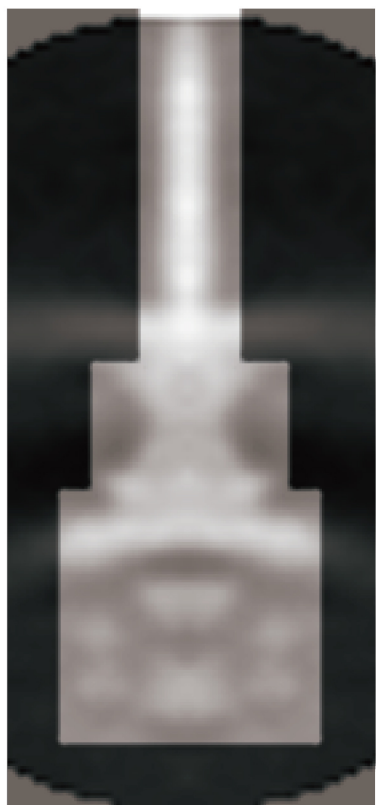
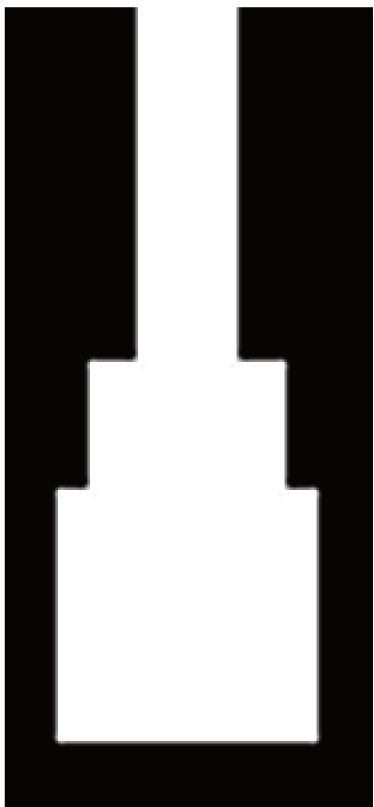
Supplementary Figure S1. Masks for alignment and classification. (a) *Top*, tight mask for alignment. *Bottom*, the mask layered on averaged vT3SS. (b) *Top*, cytoplasmic mask for classification. *Bottom*: the mask layered on averaged vT3SS.

Supplementary Figure S2. Gold-standard FSC curves of NC and HBB in *Shigella flexneri*/*Salmonella* Typhimurim. *Top*, two FSC curves show resolution of NC in *Shigella* at 0.143 criterium with C1 (*red*) and C6 symmetry (*blue*). *Middle*, two FSC curves shows resolution of NC in *Salmonella* at 0.143 criterium with C1 (*red*) and C6 symmetry (*blue*) *Bottom*, two FSC curves shows resolution of HBB in *Salmonella* at 0.143 criteria with C1 (*red*) and C6 symmetry (*blue*).

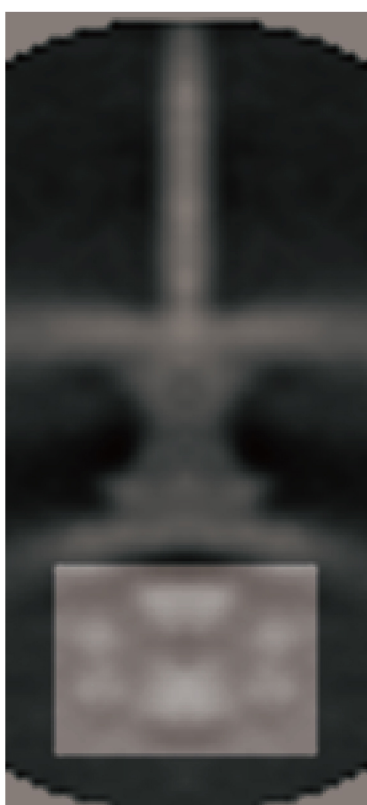
Supplementary Figure S3. Subtomogram averaging and classification of

Hook Basal Body (HBB) of *Salmonella* with C1 and C6 symmetry. (a, b) Central and cross sections of original subtomogram average with C1 and C6 symmetry **(a)** and three classes **(b)** *Below*, each corresponding cross section at height indicated by the dashed yellow line. The number of particles for each class is given on the left of each image. **(c)** Side and bottom views of 3D surface rendering of HBB. Scale bar indicates 10 nm.

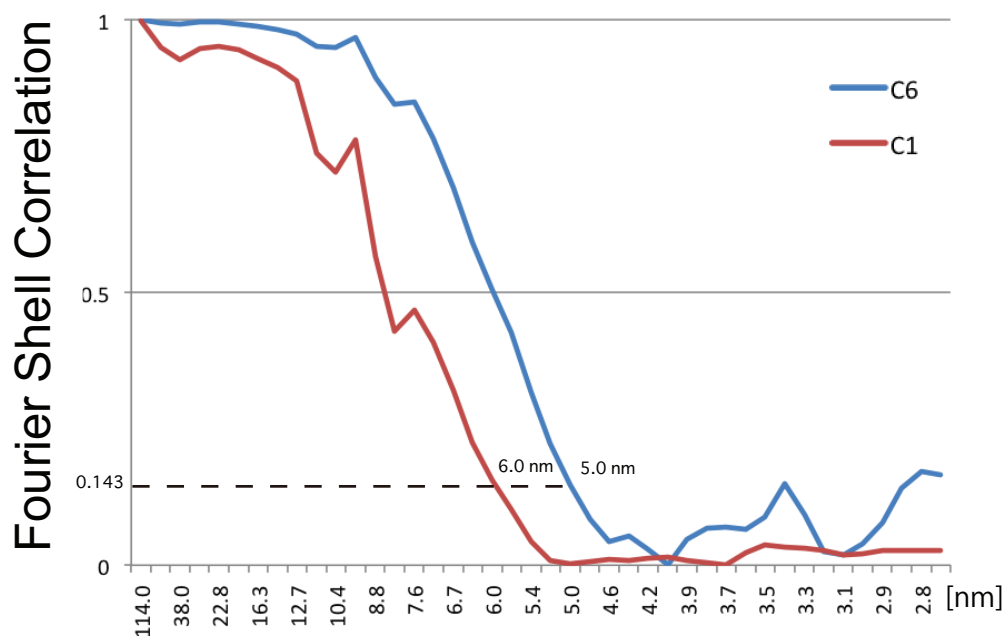
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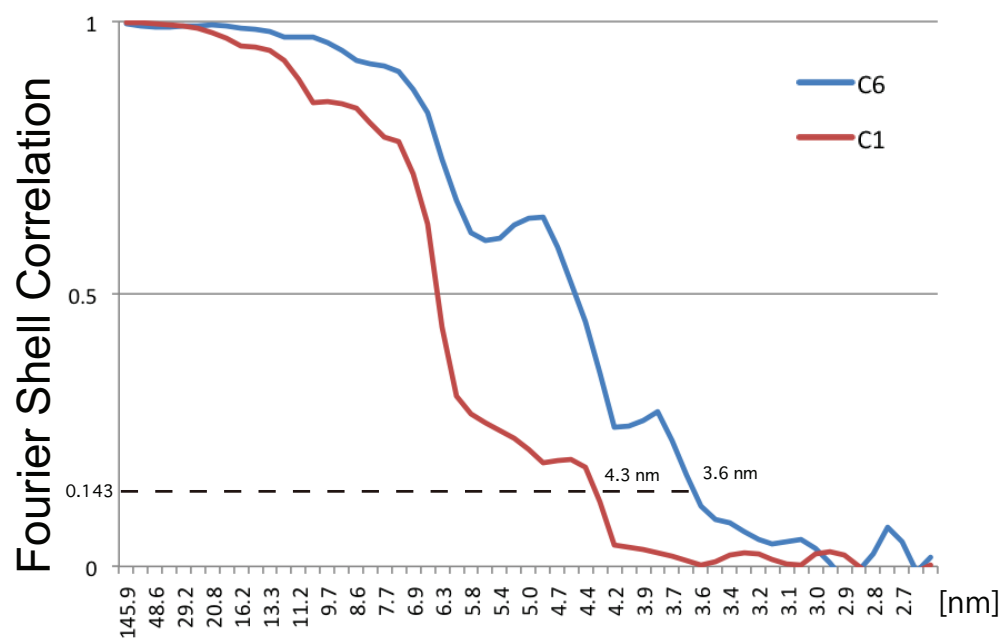
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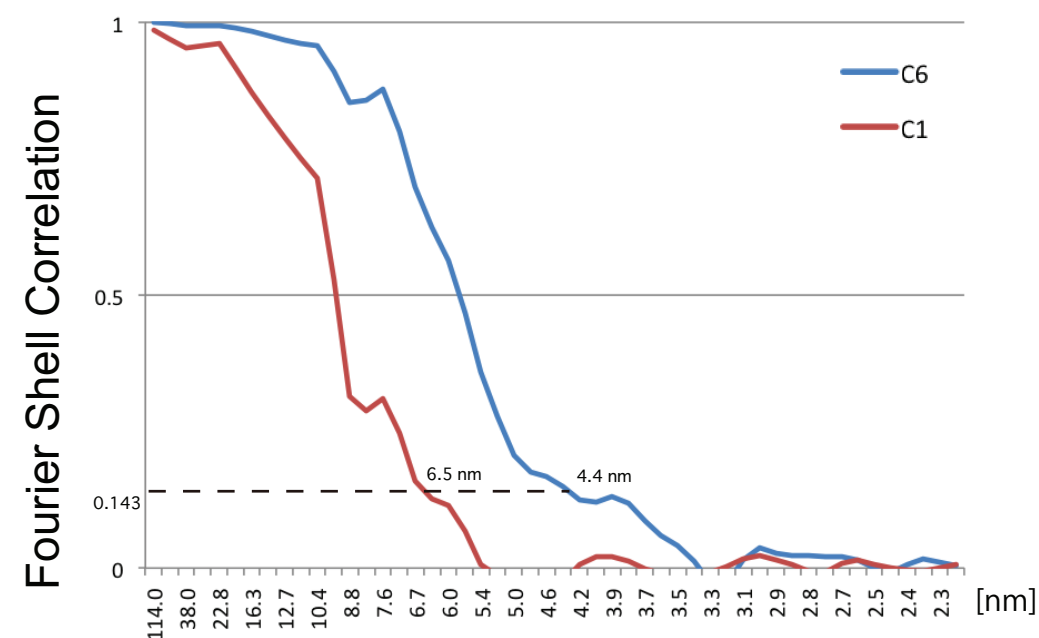
FSC curve of *Shigella* NC



FSC curve of *Salmonella* NC



FSC curve of *Salmonella* HBB



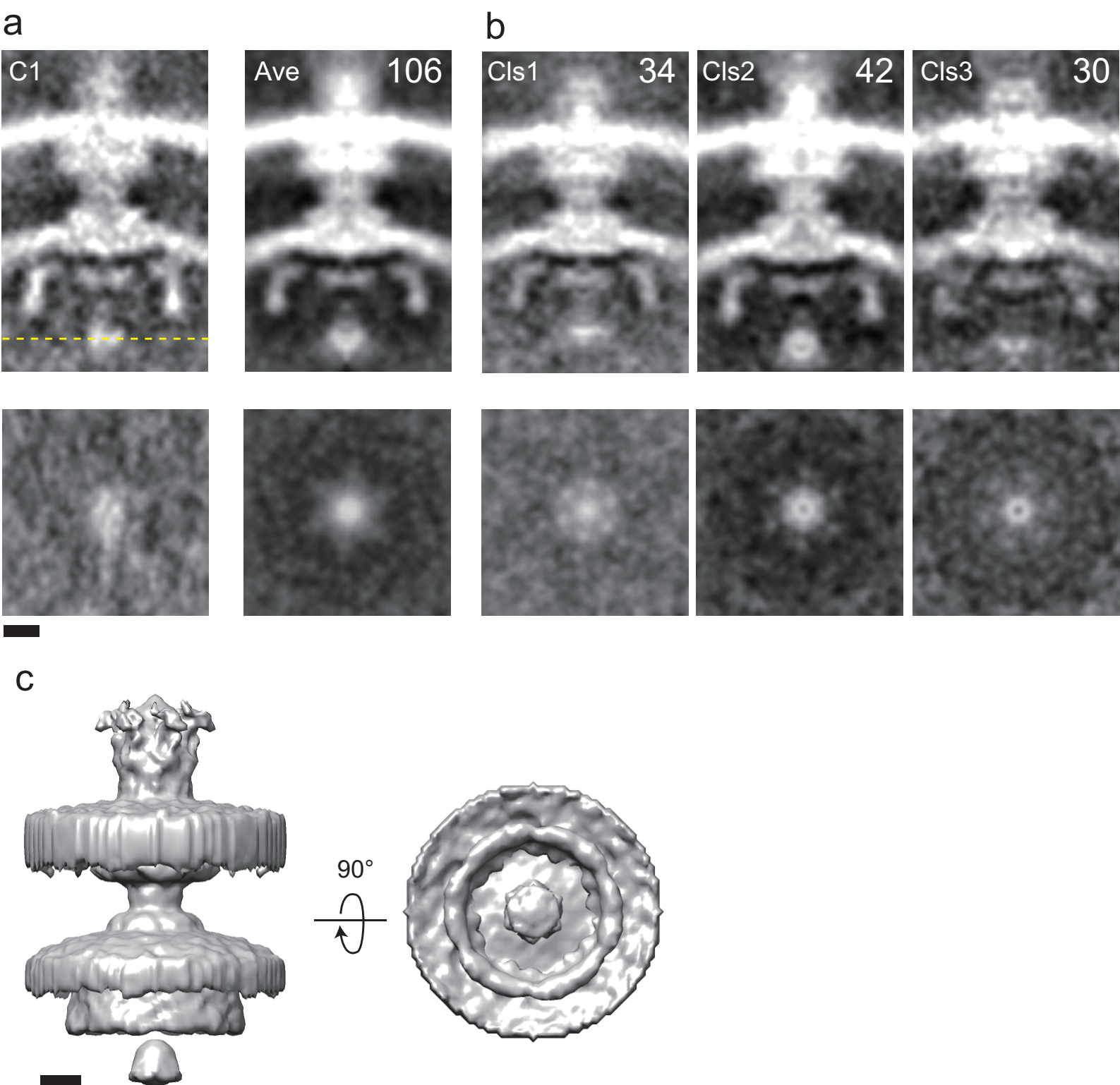


Figure S3